# RECENT EXPERIENCES WITH *Salmonella* sp. AT THE DENVER ZOOLOGICAL GARDENS

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## INTRODUCTION

This paper will present the basic principles utilized during the investigation in four recent *Salmonella* sp. outbreaks including principles of epidemiology, primary isolation techniques, and biotyping. The outbreaks occurred in four different areas of the zoo. The species involved in the outbreaks were black rhinoceroses (*Diceros bicornis*), colobus monkeys (*Colobus quereza kikuyuensis*), maned wolves (*Chrysocyon brachyurus*) and Komodo Dragons (*Varanus komodoensis*). There will be a brief discussion concerning the special problems that reptiles present in relation to salmonellosis.

## MATERIALS AND METHODS

*Salmonella* sp. is an enteric gram-negative rod, non-lactose fermenter and H2S positive bacteria that is generally associated with infections of the gastrointestinal tract, but can be found in other parts of body as well (Murry 1995). It has been reported in bovine, porcine, equine, canine, feline, avian, primate (including man), and reptilian outbreaks (Carter 1993). Because there are so many species and serotypes, the disease can present itself from a light enteritis of short duration to a severe, debilitating and life-threatening disease (Murry 1995). Presently, there is a great deal of interest in reptile-associated infections, since reptiles are becoming a very popular pet and present a risk to the unsuspecting owners (Frye 1995).

*Salmonella* sp. is isolated by culturing. At the Denver Zoological Gardens (DZG) diagnostic laboratory, the specimen is plated onto Blood Agar (TSA w/5% Sheep Blood), MacConkey Agar, and Hektoen Agar (HE). Then the swab is placed into an enrichment broth (GN) and incubated at 35°C overnight. The enrichment broth is then subcultured onto another set of Blood, MacConkey and HE plates. On HE plates, *Salmonella* sp. appears as a colorless (green/not opaque) colony with a blackened center. As *Salmonella* sp. is intermittently shed in the feces, multiple cultures may be collected before the organism is isolated. Recovery can also be hindered by the start of antibiotic treatments. Once the organism is isolated, it can be identified by one of several biochemical identification systems (Carter 1993, Murray 1995). In the DZG laboratory, the BBL Crystal® system (Becton Dickinson Microbiology Systems, Becton Dickinson and Company, Cockeysville, Maryland 21030 USA) is used.

There are over 2,000 closely related serovars of *Salmonellae* (Carter 1993). Therefore, it is epidemiologically important that all isolates should be serotyped. The first step of the process is to test a pure culture of the bacteria with a commercially available set of polyvalent O antisera. DZG sends isolates to the Colorado State University (CSU) Diagnostic Laboratory (CSU, Fort Collins, Colorado 80523, USA) to be confirmed and tested with the antisera. The antisera tests for the subgroups A through I is by slide agglutination. Serotyping is done by the Kauffmann-White antigenic schema at National Veterinary Services Laboratories (NVSL) Ames, Iowa 50010, USA. This is based on O or somatic antigens that are located on the body of the bacterial cell and are heat stable. The H antigens are flagellar antigens and are heat labile. Some of the H antigens may be diphasic. Both flagellar antigens must be identified to have a complete serotype. This involves phase changing techniques and should be done by a reference laboratory.

## CASE REPORTS

I. In November 1991, there was an outbreak of *Salmonella* sp. in the Kikuyu Colobus Monkey collection at DZG. The index case was a two-year-old male Colobus. The animal was brought to the hospital with weakness and fluid in the lungs. It was dead the next morning. Cultures of the lungs taken at necropsy produced a pure culture of *Salmonella enteritidis* Group D1. Before the results of this culture returned from CSU, a two-year-old Colobus female was brought to the hospital with the same symptoms, was treated and after several weeks recovered. A juvenile male became ill and was brought with the mother to the hospital. The juvenile died during evaluation and the mother was housed in the hospital for treatments. *Salmonella* sp. group D1 was isolated from juvenile's necropsy lung cultures. A baby male was born one month after the index case and abandoned by the mother. The baby was brought to the nursery for hand-raising. Two days later, the mother died. Two cultures were done on the mother; one breast milk and one rectal culture. The breast milk came back negative, but the rectal culture reveled *Salmonella* sp. Group D1. Trying to located the source of the infection, five mice were trapped from the building and intestinal loops were sent to CSU for culturing. When the outbreak was over, there were three dead, one infected by Salmonella but recovered, and six unaffected animals.

II. In July 1994, three Maned Wolves in our collection displayed signs of lethargy, diarrhea, and anorexia. After the DZG laboratory did several fecal cultures with negative results, fecal cultures were submitted to CSU. Two weeks after initial onset, CSU reported a positive fecal culture on the female for *Salmonella* sp. group C2. This was serotyped by National Veterinary Services Laboratories of Ames, Iowa (NVSL) as a serotype Kentucky. Mice fed to the Maned Wolves were cultured and proved to be negative. One month later, one male Maned Wolf was euthanized after losing more than half his body weight. The *Salmonella* was never recovered from any other cultures.

III. In November 1995, one of the female black rhinoceroses at DZG was reported to be lethargic and had a swollen jaw. Two weeks later, the rhinoceros was still lethargic and now inappetent. Several mice in the building were trapped and ligated intestinal loops were sent to CSU for culturing. The report came back with mixed growth of several enteric bacteria, but no *salmonella* sp. was isolated. Three weeks after the initial presentation, the rhinoceros was immobilized and cultures of a nasal ulcer and an abscess on the jaw were obtained. In addition, blood was drawn for CBC, chemistries, vitamin E levels, Leptospiridia titers and research requests. The culture of the nasal ulcer yielded: Moderate growth of a hemolytic *Streptococcus* sp. not Group A, moderate growth of *Acinetobacter baumanii*, and on subculture *Salmonella arizonae* was isolated. The *Salmonella arizonae* was a surprise finding as this is not a common nasal flora. *Salmonella arizonae* is, however, often cultured from feces of reptiles. The isolate was sent to CSU for confirmation. They also identified it as *Salmonella arizonae* and sent the isolate to the NVSL for serological typing. The serotyping of this organism was 44:Z4,Z32(Arizona). Fecal cultures were negative for *Salmonella* sp. As the rhinoceros was on antibiotics, the recovery of the *Salmonella* sp. could have been masked. One month after presentation, the rhinoceros had a purulent discharge from the vagina. A gram stain of this material revealed 4+ polymorphonuclear leukocytes (PMNs) with intracellular bacteria. When cultured, a heavy growth of a pure culture of *Salmonella arizonae* grew. At this time, concerned that animal was septic with *Salmonella arizonae*, we did a Disk Diffusion Sensitivity - the organism was sensitive to Baytril, Ceftriaxone, Amikacin, and SXT (Finegold 1986). Two days later, the rhinoceros was immobilized again antibiotic treatments and blood collecting. Two sets of blood cultures were obtained. The blood cultures were subcultured and on day seven, the aerobic bottle was positive for *Salmonella arizonae*. The rhinoceros had a *Salmonella* sepsis. Four months after presentation, the female was found dead in its enclosure. On necropsy, several cultures were taken, the most interesting was from the pleura space that grew an organism most closely resembling *Pseudomonas putrifaciens* and a heavy growth of *Salmonella arizonae*. This isolate would type as 44:Z4,Z32(Arizona).

During this time, we started to try to quarantine the area from the other animals in the building as well as prevent the spread of the *Salmonella* to the Pachyderm zookeepers. Roccal®-D (The Upjohn Company, Kalamazoo, Michigan 49001, USA) footbaths were placed outside each enclosure and paper

coveralls (Keppler® Keppler Company, Guntersville, Alabama 35976) and latex gloves were provided to reduce the risk of transmission.

Fecal cultures were collected on all of the rhinoceroses in the building. There were three males and two female black rhinoceroses. In December, a one-year-old black rhinoceros had a positive fecal culture for a light growth of *Salmonella arizonae*. The serotyping of this isolate: Untypable (rough O:ZA, Z32 - Arizona).

We also isolated a light growth of *Salmonella arizonae* on a 22 year old male black rhinoceros. This isolate was serotyped and the report came back: Untypeable (rough O:ZA, Z32 - Arizona). This rhinoceros had intermittent loose stools and became inappetant. Antibiotic treatments were started. Two weeks later, it was no longer drinking. The rhinoceros was immobilized and blood was collected for CBC, chemistries, Leptosporidia titers, Vitamin E and A levels, and research projects. Two sets of blood cultures were negative for *Salmonella*. The animal progressed poorly and was euthanized three weeks later. Cultures were taken at necropsy of the lung and cecum. No *Salmonella* species were isolated.

Several years previously, Tokay Geckos had been introduced into the Pachyderm Building in an attempt to help control insect populations. As these reptiles might be another source of *Salmonella arizonae*, traps were set and two geckos were collected and cultured. One culture grew *Salmonella* sp. Group D and the serotype was Eastbourne.

IV. In January 1996, the Colorado Department of Public Health and Environment (CDPHE) informed the zoo that they believed they had traced a public outbreak of *Salmonella enteritidis* to an event held at our Tropical Rainforest building. An outbreak is defined as the time the disease appears in a single host species until it disappears from that population (Flectcher 1982). There were seven index cases from several hospitals in the Denver metro area. The original investigations led the Health Department to an event called "Dragon Days" - a temporary exhibit of the komodo dragons. It took place in the lobby of our Tropical Building. This special exhibit was held for one week in January. As soon as we were notified of the outbreak, the veterinary staff requested to have eight reptiles in Tropical Discovery cultured for the isolation of *Salmonella* spp. The reptiles were the four komodo dragons and four reptiles used for public display by the Education Department adjacent to the event. Cloacal swabs were obtained on all the animals. In addition, skin cultures were collected, a moist swab was rubbed over the tail area behind the vent on the four komodo dragons. Fecal cultures were obtained the next day from those reptiles that defecated. Cultures were repeated one day later by the CDPHE. The DZG diagnostic laboratory isolated several serotypes of *Salmonella* spp. including the serotype that the CDPHE had implicated in the outbreak, *Salmonella enteritidis* with a unique rough morphology. In the 18 cultures that were collected during the outbreak, the DZG results matched CDPHE. Several other serotypes of *Salmonella* were isolated from the reptiles including *Salmonella thompson*, *Salmonella blukwa*, and three different serotypes of *Salmonella arizonae*. Cultures were taken from a water monitor skin that was used as a visitor hands on exhibit as well as from two feeder rats. These cultures were negative.

One week later, two investigators from the Center of Disease Control (CDC) (Report to be published by Dr. Freidman at a later date). As part of their investigation, they concluded that there was indirect transmission of the *Salmonella enteritidis* from the temporary wooden barrier (personal communication).

## DISCUSSION

Epidemiology is the study of the frequency, distributions and determinants of health and disease in populations. There are two approaches in epidemiology: descriptive epidemiology and analytic epidemiology. Descriptive epidemiology is the description of what the disease is: who, when, and where it occurs. Analytic epidemiology is the collection and analysis of data to test a hypothesis: why the disease occurs. Recognizing the disease state is an important element of the study of

epidemiology. In the past, most work in epidemiology has pertained to infectious diseases and was linked closely to the science of microbiology (Flectcher 1982, Gillespie 1982, Martin 1987).

The purpose of epidemiology is the prevention of disease. This involves primary, secondary and tertiary prevention of disease. Primary prevention is the practice that attempts to prevent exposure of the animal to the causative agent of the disease. These practices include quarantine, hygiene, vaccination, and dietary needs. Secondary prevention is the practice used to detect the disease process as early as possible. Screening tests, preliminary examinations, and careful observations of changes from "normal" behaviors are all means of disease detection. The methods of spreading infectious disease often complicated. Infectious disease can be transmitted by direct contact or fomites as well as vectors such as soil, food, water, air, and arthropods. Sometimes a weakening of an animal's natural immune system can lead to infections from normal flora. When trying to determine the source of an outbreak, it is important to keep in mind these methods of transmission. Often, when dealing with infectious bacterial outbreaks, sampling the food, water, and soil found in an animal's enclosure can lead to identifying the source of the infection. Some of the methods of preventing the spread of the disease including isolation, footbaths, protective clothing, and culling. Tertiary prevention is therapy or treatment of the disease. These include practices that increase the survivability of the animal: drugs, surgery, and supportive care (Flectcher 1982, Gillespie 1982, Martin 1987).

These case reports are an example of descriptive epidemiology. The careful reporting of the case itself can often lead to answers. After several cases have been reported, an investigator can review that material and look for common occurrences and perhaps determine the causative agent. An institution should support the sharing of information with other institutions, governmental agencies, and colleagues through the participation in conferences, professional organization, and publishing of papers.

Analytic epidemiology is the purpose of most bacterial culturing. Why did this disease occur? Often it is the isolation of a pathogenic bacterium that will lead to the recognition of an outbreak in a certain animal population. In zoo settings, because of the small populations, an outbreak can be defined as two or more animals with the same pathogen.

There are examples of each of the practices of epidemiology in the case reports. Primary practices from the cases include daily cage cleaning, good food quality and permanent footbaths like around the colobus monkey exhibit. Secondary practices include the exam of the animal as soon as the disease presented, removal of the animal to the hospital (monkey and maned wolf cases), isolation of the area using protective clothing and footbaths (black rhino case) and screening of the fecal of other animals in the building (black rhino case). Tertiary prevention examples include the treatment and support of the animals after they became sick (all cases) and the supportive care that leads to the recovery of some animals. These practices will lower the incidences of disease, however, even with the strictest preventative measures, there were still will be outbreaks of infectious disease.

There are several important points to remember when dealing with Salmonella. *Salmonella* is shed intermittently, so several cultures should be taken before ruling out the organism as a causative agent (Frye 1995). Reptiles can asymptomatic carriers of *Salmonella.* It has been recommended not to try to clear these animals by use of broad spectrum antibiotics. This practice can lead to antibiotic resistant strains (Cambre 1980). Hand washing after handling a reptile is a must (Frye 1995). *Salmonella* is a zoonotic disease and can be transmitted by direct and indirect contact (CDC 1992).

Serotyping is extremely important for identifying different types of *Salmonella* sp. Grouping only is not sufficient. During a series of outbreaks, there maybe several different serovars indicating that the outbreaks are unrelated or the serovars could all be the same indicating that only one organism is causative (Finegold 1986).

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